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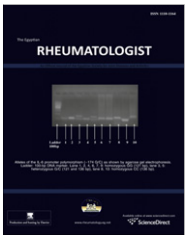

ORIGINAL ARTICLE

Increased expression of soluble fractalkine (CX3CL1) in systemic sclerosis – possible role in vascular inflammation

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KEYWORDS

Systemic sclerosis;
Soluble fractalkine (CX3CL1);
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Abstract *Introduction:* The pathogenesis of systemic sclerosis (SSc) involves interplay between obliterative vasculopathy in multiple vascular beds, inflammation, autoimmunity and progressive fibrosis. Vascular injury and activation are the earliest and possibly primary events in the pathogenesis of SSc.

Aim of the work: To determine levels of serum soluble fractalkine (sFKN) and its receptor CX3CR1 in peripheral blood mononuclear cells (PBMCs) in systemic sclerosis (SSc) patients and healthy controls. In addition, to assess any possible association between sFKN and clinical features of SSc.

Patients and methods: Serum levels of soluble fractalkine (sFKN, CX3CL1) assessed by enzyme linked immunosorbent assay (ELISA) and expression of its receptor (CX3CR1) on peripheral blood mononuclear cells by flow cytometric analysis, were measured in 18 systemic sclerosis (SSc) patients

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and 15 age and sex matched healthy controls. The degree of skin involvement was estimated by modified Rodnan skin thickness score (mRSS), pulmonary involvement was assessed in all patients by high resolution computerized tomography (HRCT) and pulmonary function tests (PFTs).

Results: Serum sFKN levels and expression of its receptor CX3CR1 were significantly elevated in SSc patients than in healthy controls ($P < 0.0.05$). SSc patients with pulmonary fibrosis had sFKN levels three times higher than those without PF. Serum sFKN correlated inversely with forced vital capacity of lungs (FVC%) but correlated positively with severity of pulmonary fibrosis, extent of skin fibrosis (mRSS), pitting scars, skin ulcers, anti topo-isomerase1antibody and CRP.

Conclusion: Serum sFKN may play an important role in the pathogenesis of SSc, including tissue inflammation and vascular injury, hence, its measurement may be a useful serologic marker for the diagnosis and follow up of pulmonary and skin complications. So strategies to target $CX3CL_1$–$CX3CR_1$ interaction could provide a new therapeutic approach in SSc, potentially by blocking endothelial cell injury, leucocyte infiltration, and vascular injury.

1. Introduction

Systemic sclerosis (SSc) is an autoimmune disease characterized early by vasculopathy and subsequently by varying the degree of fibrosis in skin, lungs and other tissues. The presence of fibrosis is the hallmark of this disease [1].

The pathogenesis of SSc involves an interplay between obliterative vasculopathy in multiple vascular beds, inflammation, autoimmunity and progressive fibrosis [2,3]. Vascular injury and activation are the earliest and possibly primary events in the pathogenesis of SSc [4].

Endothelial cell (EC) dysfunction is a common feature in several immune-mediated inflammatory diseases, including vasculitis 4. Activated ECs amplify and perpetuate inflammatory processes by expressing and secreting a variety of cytokines, chemokines, cell-mediated molecules, and other inflammatory molecules. Moreover, interaction between ECs and invading mononuclear cells is essential for the progression of vasculitis [5].

Pulmonary complications are one of the most challenging complications of systemic sclerosis [6]. Pulmonary fibrosis (PF) develops in more than 50% of SSc patients and is the major cause of death [7].

To assess the activity of PF, previous studies have identified several important signs, including patchy areas with a ground-glass or reticular appearance on high-resolution computed tomography (HRCT) [7]. Recently HRCT plays a key role in determining the prognosis of patients with SSc [8]. However, easier, less-invasive, serologic markers would be helpful for closely monitoring the activity of PF in SSc patients.

For a considerable time, skin involvement in SSc was regulated as a surrogate marker for internal organ involvement [9]. However, recently it was shown that improvement of skin sclerosis, spontaneously or as a result of treatment, does not necessarily reflect improvement of organ involvement [10]. Thus the failure of skin fibrosis to serve as a surrogate marker for severe manifestation in SSc stressed the importance of finding better and more predictive markers for life threatening disease complications.

The chemokine fractalkine (FKN; CX3CL1) is a unique member of CX3C chemokine subfamily. In contrast to other chemokines, it exists in two forms, each mediating distinct biological actions [11]. The membrane-anchored protein, which is primarily expressed on the inflamed endothelium, serves as an adhesion protein promoting the retention of monocytes and T cells in inflamed tissue. The soluble form resembles more of a conventional chemokine and strongly induces chemotaxis. Both chemotaxis and adhesion are mediated by the G protein-coupled receptor CX3CR1 [12,13].

sFKN localized on the endothelial cells not only promotes leucocyte activation but, unlike other chemokines, can also mediate each individual step of the leucocyte adhesion cascade, including capture, rolling, and firm adhesion [14,15]. Accumulating evidence suggests that sKN–CX3CR1 interaction might contribute to the development of vascular injury and inflammatory diseases, by recruiting activated leucocytes [15–17].

The levels of CX3CL1 expression by ECs are low in healthy individuals in the absence of an inflammatory insult, but at sites of inflammation, the levels of both the membrane-bond and secreted forms are greatly up regulated by inflammatory cytokines [11]. Thus, CX3CL1 appears to possess immunoregulatory properties that affect inflammatory and immune cell–EC interactions and inflammatory responses at inflamed sites.

Indeed, investigations by several groups have implicated CX3CL1 in a variety of inflammatory disorders, including glomerulonephritis, RA, systemic sclerosis and SLE [18,12].

2. Aim of the work

The aim of the present study was to determine the serum levels of sFKN (CX3CL1) and its receptor (CX3CR1) on peripheral blood mononuclear cells, In addition, to assess any possible association between sFKN and clinical features of the disease.

3. Patients and methods

A total of 18 non smoking female SSc patients who fulfilled the criteria proposed by the American College of Rheumatology for SSc [19] were enrolled in this study. The mean age of the patients was 37.3 ± 4.2 years and the mean disease duration was 7.8 ± 5.4 years. Disease duration was calculated from the time of the first clinical event: Raynaud's phenomenon, the first presence of skin involvement or the first presence of organ involvement .Informed consent was obtained from all patients.

Fifteen age and sex-matched healthy volunteers served as controls for fractalkine, its receptor CX3CR1 and C-reactive protein (CRP) measurement.

3.1. Exclusion criteria

Cigarette smoking or recent history of infection or other systemic inflammatory diseases.

3.1.1. Clinical assessment

Complete medical histories were obtained, physical examinations, laboratory tests (ESR, CRP, kidney function tests, complete urine analysis) and immunological tests (anticentromere antibody, anti-topoisomerase I antibody and ANA) were conducted for all patients at their first visit with evaluations especially for pulmonary function during follow up visits. Skin thickness was scored according to the modified Rodnan skin thickness score (mRSS) by summing the skin thickness measurements as determined by palpation on a 0–3 scale in 17 body areas (range 0–51) [20].

Organ system involvement was defined as described previously [21,22] lung = bibasilar fibrosis on chest radiography and high resolution computed tomography; isolated pulmonary hypertension, color Doppler echocardiography for the definition of PAH. A pulmonary artery systolic pressure (PASP) >35 mm Hg was used to define PAH; esophagus = hypomobility shown by barium radiography; joint = inflammatory polyarthralgia or arthritis; heart = pericarditis, congestive heart failure, or arrhythmias requiring treatment; kidney = malignant hypertension and rapidly progressive renal failure without any other explanation; and muscle = proximal muscle weakness and elevated serum creatine kinase concentration.

Pulmonary fibrosis (PF) was defined as bibasilar interstitial fibrosis on chest high resolution computed tomography (HRCT). Pulmonary fibrosis was graded by radiologist according to the extent of involvement, grade l: basal and subpleural, grade 2: basal and mid-zonal, grade 3: extensive fibrosis ± bronchiectasis. In addition, pulmonary function tests, including vital capacity of lungs (VC%) and forced expiratory volume in the first, second FEV1, FEV1/FVC (FEV1%)were also evaluated to examine the severity of PF. Restrictive lung disease was diagnosed if FVC, percent of predicted value was <80% with FEV1/FVC actual value in the normal range (more than 80%).

Restrictive lung disease was classified into mild, moderate and severe according to the FVC percent of predicted value as follows: mild restriction if FVC was 79–70%, moderate restriction 69–50%, and severe restriction if FVC was less than 50%. Obstructive pattern was diagnosed if FEV1/FVC actual value <80% [23].

Demographic and clinical characteristics of SSc patients are shown in (Table 1).

3.2. ELISA for sFKN

Human sFKN levels were measured in serum samples by ELISA according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA), using 96 well polystyrene plates coated overnight at 25 °C with 2 mg/ml of purified goat antihuman FKN IgG. Briefly, after being washed, plates were blocked for 1 h at 20 °C with phosphate buffered saline containing 1% bovine serum albumin and 5% sucrose. Recombinant human FKN and serum samples were added in triplicate, and the plates were incubated for 2 h at 20 °C. After further washing, plates were incubated with biotinylated goat antihuman FKN Ab (250 ng/ml) for 2 h at 20 °C and then with streptavidin-peroxidase for 1 h at 20 °C. Samples were developed with 0.1 ml/well of tetramethylbenzidine substrate diluted in citrate–phosphate buffer. Reactions were stopped by adding 1 M H_2SO_4 and absorbance was read at 450 nm.

3.3. Flow cytometric analysis

Fractalkine receptor CX3CR1 expression was studied on T-lymphocyte subpopulations of whole blood samples. The Tow-color analysis was performed with a combination of FITC conjugated anti-CX3CR1 (Medical and Biological Laboratories Corp, UK) and phycoerythrin conjugated anti-CD4 (Coulter Corp, Miami, FL), anti-CD8 (Coulter Corp), anti-CD14 (Coulter Corp), or anti-CD16 monoclonal Abs (Coulter Corp). The blood samples were stained at 4 °C with a predetermined optimal concentration of the test monoclonal Ab for 20 min, as previously described [18]. Blood erythrocytes were lysed after staining with the Coulter whole blood immunolyse kit as detailed by the manufacturer (Coulter Corp). Cells were washed and analyzed with an FACS Caliber flow cytometer (BD PharMingen, San Diego, CA). Positive and negative populations of cells were determined with unreactive isotype matched monoclonal Abs (Coulter Corp) as controls for background staining.

Statistics. Data were analyzed using SPSS version 11.5 Descriptive statistics were done by number and percent as well as mean and SD. Unpaired Student's *t*-test was used for comparison of frequencies, and Spearman's rank correlation coefficient was used to examine the relationship between two continuous variables. Values of $P < 0.05$ were considered significant.

4. Results

4.1. Serum sFKN levels in SSc patients and healthy controls

As shown in Table 2 and Fig. 1 SSc patients have significant elevation of sFKN levels than healthy controls (468 ± 160 vs 87 ± 20 pg/ml, $P < 0.01$).

4.2. Expression of CX3CR1 on PBMCs in SSc patients and healthy controls

As shown in Table 2 and Fig. 2, the raised levels of CX3CL1 in SSc patients were accompanied by an increase in its receptor CX3CR1 on PBMCs. Regarding T cell subtypes CD_4^+ T cells, CD_8^+ T cells and CD_{14}^+ T cells, there were significant differences between SSc patients and healthy controls (5.8 ± 1.1, 6.9 ± 2.1 and 8.2 ± 3.3 vs 2.1 ± 0.7, 2.6 ± 1.5 and 3.2 ± 1.9, $P < 0.05$), respectively. But there was no significant difference regarding CD_{16}^+ T cells between SSc patients and healthy controls (14.6 ± 5.6 vs 10.1 ± 5.6 $P > 0.05$).

Table 1 Demographic and clinical characteristics of SSc patients.

	Patient characteristics (n = 18)	Values
Continuous variables (mean + SD)	Age	37.3 + 4.2
	Disease duration (years)	7.8 + 5.4
	Modified Rodnan skin score	26.7 + 2.3
Categorical variables [n(%)]	Pitting scars or skin ulcers	13(72%)
	Contracture of phalanges	16(89%)
	Diffuse pigmentation	11(61%)
	Raynaud's phenomenon	18(100%)
	Pulmonary fibrosis on (HRCT)	11(61%)
	FVC <80%	11(61%)
	Arthritis	7(37%)
	Dysphagia	18(100%)
	Heart (pericarditis, arrythmia)	4(22%)
	Anticentromere antibody	5(28%)
	Anti-topoisomerase1 antibody	16(89%)
	CRP	9(50%)

FVC: forced vital capacity of lungs.
CRP: C reactive protein.

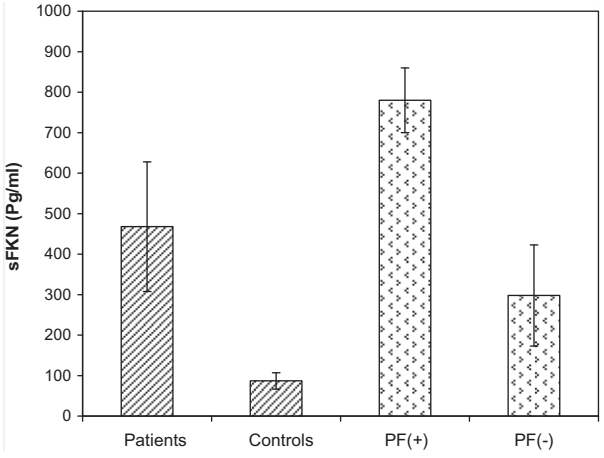

Figure 1 Serum levels of sFKN among SSc patients and controls. Also among patients with PF(+) and patients without PF(−).

4.3. Clinical and laboratory data of SSc patients

Raised serum sFKN levels were seen in 10/18 (56%) of all patients, out of them 90% had pulmonary fibrosis with a significant difference between them and control group, $P < 0.05$ (Table 3). SSc patients with PF had sFKN levels three times

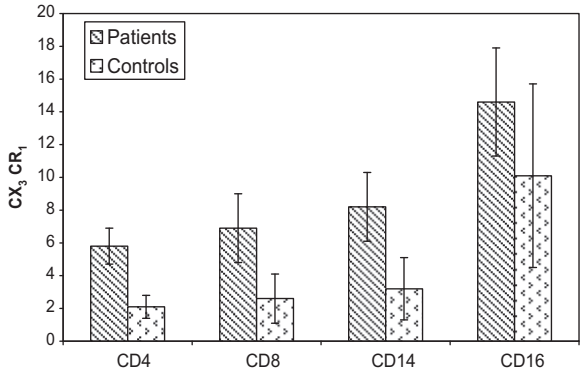

Figure 2 Expression of CX_3 CR_1 in SSc patients and controls.

Table 2 Serum sFKN (CX3CL1) and expression of its receptor (CX_3CR1) in SSc patients and controls.

sFKN (pg/ml)	Patients (n = 18)	Controls (n = 15)	P <0.05
	468 ± 160	87 ± 20	
CX_3CR_1 expression			
CD4	5.8 ± 1.1	2.1 ± 0.7	<0.05
CD8	6.9 ± 2.1	2.6 ± 1.5	<0.05
CD14	8.2 ± 3.3	3.2 ± 1.9	<0.05
CD16	14.6 ± 5.6	10.1 ± 5.6	>0.05

Table 3 Clinical and laboratory data in SSc patients having raised or normal sFKN levels.

		Raised sFKN (n = 10)	Normal sFKN (n = 8)
Continuous Variables (mean ± SD)	Age	35.12 ± 11.90	40.57 ± 11.17
	Disease duration (years)	8.40 ± 7.09	7.40 ± 6.12
	Rodnan skin score	35.9 ± 10.99*	21.1 ± 7.19
Categorical variables [n(%)]	Pitting scars or skin ulcers	7(70%)*	5(36%)
	Contracture of phalanges	9(90%)	7(88%)
	Diffuse pigmentation	6(60%)	5(63%)
	Raynaud's phenomenon	10(100%)	8(100%)
	Pulmonary fibrosis on (HRCT)	9(90%)*	2(25%)
	FVC <80%	9(90%)*	2(25%)
	Arthritis	4(40%)	3(38%)
	Dysphagia	10(100%)	8(100%)
	Anticentromere antibody	3(30%)	2(25%)
	Anti-topoisomerase antibody	7(70%)*	3(38%)
	CRP	7(70%)*	2(25%)

* $P < 0.05$ vs SSc patients with normal sFKN levels.

Table 4 Correlations between serum sFKN and studied variables in SSc patients.

Variables	*r*	*P*
Age	0.210	>0.05
Disease duration	0.123	>0.05
FVC%	−0.916	<0.05
Pulmonary fibrosis	0.762	<0.05
Rodnan skin score	0.661	<0.05
Pitting scars or skin ulcers	0.452	<0.05
Anti-topoisomerase antibody	0.792	<0.05
CRP	0.541	<0.05

higher than those without PF (780 ± 80 pg/ml vs 298 ± 125 pg/ml, $P < 0.05$), respectively (Fig. 1). SSc patients with raised sFKN levels more frequently had high mRSS, pitting scars, skin ulcers, decreased FVC%, anti-topoisomerase1 antibody and CRP than those with normal sFKN levels $P < 0.05$ (Table 3).

4.4. Correlation between CX3CL1 levels and studied variables in SSc

Serum sFKN levels correlated inversely with FVC% in SSc patient ($r = -0.916$, $P < 0.05$), but there was a significant positive correlation between sFKN and pulmonary fibrosis ($r = 0.762$, $P < 0.05$), modified Rodnan skin score ($r = 0.661$, $P < 0.05$), pitting scars or skin ulcers ($r = 0.452$, $P < 0.05$) anti-topoisomerase 1antibody ($r = 0.792$, $P < 0.05$) and CRP ($r = 0.541$, $P < 0.05$) (Table 4).

Thus, sFKN levels not only correlated with the extent of skin sclerosis but also with the severity of PF in SSc patients.

5. Discussion

The etiology of SSc is subject to ongoing research, as the precise events that underlie the development of this disease remain unclear. The pathogenesis is known to involve endothelium, epithelium, fibroblasts, innate and adaptive immune systems and their component immunological mediators. Endothelial cell dysfunction may be the initiating factor, but the precise triggering event(s) remain elusive. Vasculopathy shows similarities in different organs (e.g. pulmonary hypertension, renal disease and digital tip ulcer).Serum sFKN is a potent mediator of vasculopathy, and hence represents a highly relevant target for intervention of vascular features in SSc [24].

In the present study we show that SSc patients are characterized by markedly increased serum levels of CX3CL1, accompanied by the enhanced expression of its corresponding receptor, CX3CR1 on peripheral blood mononuclear cell (PBMC) than in healthy controls ($P < 0.005$, Table 2). These findings suggest that enhanced sFKN–CX3CR1 interaction contributes to the disease process. These results are in accordance with [15,11,18] who concluded that raised sFKN promotes CX3CR1 cell infiltration into the affected tissue leading to tissue inflammation, because sFKN enhances the chemotactic activity of cells expressing CX_3CR1.

Previously, increased CX3CL1 expression has been found in various autoimmune and vasculitic disorders such as SLE and rheumatoid arthritis, potentially contributing to neuropsychiatric manifestations and synovitis, respectively 12. Studies in animal model have shown that CX3CL1, inhibition may delay the initiation and progression of lupus nephritis [25].

In this study CX_3CL_1 and its receptor CX3CR1 were significantly increased in patients with PF than in patients without PF as evidenced by HRCT ($P < 0.05$, Fig. 1), pulmonary fibrosis (PF) was significantly detected in patients with raised sFKN than in patients with normal sFKN ($P < 0.05$). Furthermore, serum sFKN was negatively correlated with FVC% of lungs (Table 4).

Consistent with our result was the study of Hasegawa et al; [18] which revealed that serum sFKN levels were significantly associated with the involvement and severity of pulmonary fibrosis by recruiting CX3CR1 + cells to the affected lung.

Serum sFKN interacts with its unique receptor, CXCR1, to effect firm adhesion of monocytes/macrophages, natural killer (NK) cells, and a subpopulation of T cells (CD8+ T cells, CD_4+ T cells) [26].

FKN exhibits efficient chemotactic activity for monocytes/macrophages, NK cells, and T cells expressing CX3CR1 [26,27]. Accumulating evidence suggests that sFKN–CX3CR1 interaction might contribute to the development of vascular injury and inflammatory diseases, by recruiting activated leucocytes [15,28].

Therefore, sFKN may have an important role in the induction and/or development of PF in SSc patients by recruiting CX3 CR1 to the affected lungs [18]. The elevated sFKN levels (in microscopic polyangitis patients and in all systemic vasculitis patients) correlated positively with vasculitis disease activity (Birmingham vasculitis activity score), C-reactive protein levels, and erythrocyte sedimentation rate (ESR) [5]. Our results go hand in hand with the previous study where we found positive correlation between sFKN and CRP.

In the current study, serum sFKN levels were correlated positively with modified Rodnan skin score (Table 4). Moreover, digital ischemia and skin ulcers were found more frequently in patients with raised sFKN levels than in patients with normal serum sFKN levels.

Histological analysis of the initial stage of SSc shows the presence of perivascular infiltrates of mononuclear cells in the dermis, which is associated with increased collagen synthesis in the surrounding fibroblasts [29]. Consequently, our study showed that patients with SSc had increasing serum sFKN and its receptor CX3CR1 levels. Collectively, these observations suggest that augmented expression of sFKN abnormally recruits monocytes into the skin of SSc patients, mediating the initiation and propagation of skin sclerosis. Recently Kasama et al. [5], reported that up regulated expression of CX3 CR1 on endothelial cells and the accumulation of activated inflammatory cells would likely represent pathophysiologic events leading to skin vasculitis.

Previous findings indicated that circulating soluble proteases such as granzyme may be linked to the pathogenesis of endothelial injury in SSc [30,31]. Recently, it has been demonstrated that CX3CR1 expression defines mononuclear cells possessing high levels of intracellular perforin and granzyme [32].These previous findings suggest that sFKN regulates recruitment of cytotoxic cells through inflamed endothelium. Therefore, sFKN may have a critical role in cytotoxic cell mediated endothelium damage, which may result in a vascular injury.

In conclusion serum sFKN may play an important role in the pathogenesis of SSc, including tissue inflammation and vascular injury, and its measurement may be a useful serologic marker for the diagnosis and follow up of pulmonary and skin complications.

Recommendation: Enhanced CX_3CL_1–CX_3CR_1 interaction may be an important biologic factor in promoting endothelial injury in SSc. So, further studies will clarify if strategies to target $CX3CL_1$–$CX3CR_1$ interaction could provide a new therapeutic approach in SSc, potentially by blocking endothelial cell injury, leucocyte infiltration, and vascular injury.

References

[1] Tracy Frech, Nathan Hatton, Boaz Markewitz, et al. The vacular microenvironment and systemic sclerosis. Int J Rheumatol 2010:6. Article ID 362868.

[2] Chung L, Lin J, Furst DE, et al.,. Systemic and localized scleroderma. Clin Dermatol 2006;24(5):374–92.

[3] Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. J Clin Invest 2007;117(3):557–67.

[4] Buckley CD, Rainger GE, Nash GB, et al. Endothelial cells fibroblasts and vasculitis. Rheumatology 2005;44:860–3.

[5] Kasama T, Wakabashi K, Sato M, et al.. Prevalence of the CX3CR1/fractalkine- CX3CR1 pathway in vasculitis and vasculopathy. Translational Res 2010;155(1):20–6.

[6] Wells AU, Steem V, Valentini G. Pulmonary complications: one of the most challenging complications of systemic sclerosis. Rheumatology 2009;48:iii40–=0?>iii44.

[7] Sakkas LI, Chikanza IC, Patsoucas CD. Mechanisms of disease: the role of immune cells in the pathogenesis of systemic sclerosis. Nat Clin Pract Rheumtol 2006;2(12):679–85.

[8] Goh NS, Desal SR, Veeraraghavan S, et al. Interstitial lung disease in systemic sclerosis: a simple staging system. Am J Respir Crit Care Med 2008:177.

[9] Furst D, Khanna D, Matucci-Cerinic M, et al. Systemic sclerosis-continuing progress in developing clinical measures of response. J Rheumatol 2007;34(5):1194–200.

[10] Clements PJ, Roth MD, Elashoff R, et al. Scleroderma lung study group scleroderma lung study (SLS): differences in the presentation and course of patients with limited versus diffuse systemic sclerosis. Ann Rheum Dis 2007;66:1641–7.

[11] Umehara H, Bloom ET, Okazaki T, et al. Fractalkine in vascular biology. From basic research to clinical disease. Arterioscler Thromb Vasc Biol 2004;24:34–40.

[12] Yajima N, Kasama T, Isozaki T, Odai T, et al. Elevated levels of soluble fractakine in active systemic lupus erythematosus: potential involvement in neuropsychiatric manifestations. Arthritis Rheum 2005;52(6):1670–5.

[13] Bjerkeli V, Damas JK, Fevan B, et al. Increased expression of fractalkine (CX3CL1) and its receptor, CX3CR1, in Wegner's granulomatosis- possible role in vascular inflammation. Rheumatology 2007;46:1422–7.

[14] Fong AM, Robinson LA, Steeber DA, et al. Fractalkine and CX3CR1 mediate a novel mechanism of leukocyte capture, firm adhesion, and activation under physiologic flow. J Exp Injury 1998;188:1413–9.

[15] umehara H, Bloom E, okazaki T. Fractalkine and vascular injury. Trends Immunol 2001;22:602–7.

[16] Ruth JH, Rottman JB, Katschke Jr KJ, et al. Selective lymphocyte chemokine receptor expression in the rheumatoid joint. Arthritis Rheum 2001;44:2750–60.

[17] Feng L, Chen S, Garcia GE, et al. Prevention of crescentic glomerulonephritis by immunoneutralization of the fractalkine receptor CX3CR1 rapid communication. Kidney Int 1999;56:612–20.

[18] Hasegawa M, Sato S, Echigo T, et al. Up regulated expression of fractalkine/CX3CL1 and CX3CR1 in patients with systemic sclerosis. Ann Rheum Dis 2005;64:21–8.

[19] ARA. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581–90.

[20] Clements PJ, Lachenbruch PA, Seibold JR, et al. Skin thickness score in systemic sclerosis: an assessment of interobeser variability in 3 independent studies. J Rheumatol 1993;20:1892–6.

[21] Steen VD, powell DL, Medsger TAJ. Clinical correlations and prognosis based on serum autoantibodies in patients with systemic sclerosis. Arthritis Rheum 1988;31:196–203.

[22] Sato S, Ihn H, Kikuchi K, et al. Antihistone antibodies in systemic sclerosis: association with pulmonary fibrosis. Arthritis Rheum 1994;37:391–4, 23.

[23] Rupple GL. Manual of pulmonary function testing. 5th ed., St. Louis; 1991: p. 49.

[24] Abraham DJ, Krieg T, Distler J, et al. Overview of pathogenesis of systemic sclerosis. Rheumatology 2009;48:iii3–=0?>iii7.

[25] Inoue A, Hasegawa H, Kohno M Ito MR, et al.. Antagonist of fractalkine (CX3CL 1) delays the initiation and ameliorates the progression of lupus nephritis in MRL pr mice. Arthritis Rheum 2005;52:1522–33.

[26] Imai T, Hieshima K, Haskell C, et al. identification and molecular characterization of fractalkine receptor CXCR1, which mediates both leukocyte migration and adhesion. Cell 1997;91:521–30.

[27] Bazan JF, Bacon KB, Hardiman G, et al. A new class of membrane-bound chemokine with a CX3C motif. Nature 1997;385:640–4.

[28] Volin MV, Woods JM, Amin MA, et al. Fractalkine: a novel angiogenic chemokine in rheumatoid arthritis. Am J Pathol 2001;159:1521–30.

[29] Yanaba K, komura K, Matsushita T, et al. Serum levels of monocyte chemotactic protein -3/CCL7 are raised in patients with systemic sclerosis: association with extent of skin sclerosis and severity of pulmonary fibrosis. Ann Rheum Dis 2006;65:124–6.

[30] Matucci-Cerinic M, Kahaleh MB. Pathogenesis: vascular involvement. In: Clements PJ, Furst DE, editors. Systemic sclerosis. Baltimore: Williams & Wilkins; 1996. p. 153–74.

[31] Kahaleh MB, Fan PS. Mechanism of serum-mediated endothelial injury in scleroderma: identification of a granular enzyme in scleroderma skin and sera. Clin Immunol Immunopathol 1997;83:32–40.

[32] Nishimura M, Umehara H, Nakayama T, et al. Dual functions of fractalkine/CX3C ligand 1 in trafficking of perforin+/granzyme B+ cytotoxic effector lymphocytes that are defined by CX3CR1 expression. J Immunol 2002;168:6173–80.